

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of all claims in the application.

Listing of Claims

1. **(Currently amended)** A method of identifying a transmembrane receptor (TMR) G protein coupled receptor (GPCR) agonist, wherein the TMR-agonist (TMRA) GPCR agonist is capable of activating TMR GPCR signaling while exhibiting reduced TMR GPCR internalization ~~ever as compared to~~ a control compound, comprising the steps of:
 - (a) providing a cell comprising at least one GPCR or a modified GPCR capable of activating intracellular signaling TMR, or a biologically active fragment thereof, wherein the cell further comprises and an arrestin, or a biologically active fragment thereof capable of binding a GPCR,
 - (b) exposing the cell to at least one test compound,
 - (c) measuring [[the]] GPCR signaling at two or more points in time,
 - (d) measuring the translocation internalization of the TMR GPCR at two or more points in time,
 - (e) quantitatively determining if the TMR GPCR internalization is reduced by comparing the TMR GPCR internalization in the presence of the test compound to the TMR GPCR internalization in the presence of a control compound, and wherein the GPCR signaling is activated in the presence of the test compound as compared to TMR GPCR signaling in the absence of the test compound, agonist; and
 - (f) wherein reduced TMR a reduction in GPCR internalization in the presence of the test compound as compared to the control compound indicates the test compound is a GPCR identifies a TMR agonist capable of activating GPCR signaling while exhibiting reduced GPCR internalization.
2. **(Canceled)** The method of claim 1, wherein the TMR is a GPCR.
3. **(Currently Amended)** The method of claim 1, wherein the translocation internalization of the TMR GPCR is measured by monitoring localization of a detectable molecule bound to the arrestin or to the TMR GPCR.

4. **(Previously Presented)** The method of claim 1, wherein signaling is quantitated by measuring an intracellular effector, wherein said effector is cAMP, cyclic GMP, calcium, a lipid, phosphatidylinositol, a hydrogen ion, or an ion transport molecule.

5-7. **(Canceled)**

8. **(Currently Amended)** The method of claim 1, wherein the signaling is activated for a longer time period after stimulation by the ~~TMR~~ test compound than the length of time of activation after stimulation by the control compound.

9. **(Currently Amended)** The method of claim 1, wherein the translocation internalization of the ~~TMR~~ GPCR is measured by determining the localization of the GPCR in the plasma membrane, pits, endosomes, endocytic vesicles, or cytosol.

10. **(Currently Amended)** The method of ~~claim 2~~ claim 1, wherein the GPCR is a class A, or class B receptor.

11. **(Currently Amended)** The method of ~~claim 2~~ claim 1, wherein the GPCR is a μ opioid, β_1 AR, β_2 AR, or dopamine receptor.

12. **(Currently Amended)** The method of claim 1, wherein the translocation internalization of the ~~TMR~~ GPCR is measured by visualization of a radioisotope, an epitope tag, an affinity label, an enzyme, a fluorescent group, or a chemiluminescent group attached to the arrestin or the ~~TMR~~ GPCR.

13. **(Currently amended)** The method of claim 1, wherein the signaling is measured at the same time as the translocation internalization is measured.

14. **(Original)** The method of claim 1, wherein the cell is exposed to the compound once, and wherein the cell is not exposed a second time to the compound.

15. **(Withdrawn)** A compound discovered by the method of claim 1.

16. **(Currently Amended)** The method of claim 1, wherein the ~~TMR~~ GPCR is a rat, mouse, pig, or primate ~~TMR~~ GPCR.

17. **(Currently Amended)** The method of claim 1, wherein the method is steps (a) ~ (f) are repeated, and wherein the ~~TMR~~ GPCR used in the repeated steps repeat method is from a different species than the GPCR used in the original method steps (a) ~ (f).

18. **(Currently Amended)** The method of claim 17, wherein a test compound that is used in steps (a) – (f) a TMRA in the original method is not used a TMRA in the repeated steps repeat method, and wherein the repeat method contains a TMR from a different species.

19. **(Previously Presented)** The method of claim 1, wherein the test compound is from a combinatorial library.

20. **(Previously Presented)** The method of claim 1, wherein the signaling in the presence of the test compound is equal to or greater than the signaling in the presence of the control compound.

21. **(Currently amended)** The method of claim 1, wherein the method is repeated at different concentrations of compound to yield a dose-response dose-response curve for the signaling measurement and a dose-response dose-response curve for the translocation internalization measurement in the presence of the test compound.

22. **(Currently amended)** The method of claim 21, wherein the quantitative determination includes a comparison of the dose-response dose-response curve for the signaling measurement to the dose-response dose-response curve for the translocation internalization measurement.

23. **(Currently amended)** The method of claim 21, wherein a second dose-response dose-response curve for the signaling measurement and a second dose-response dose-response curve for the translocation internalization measurement are determined in the presence of control compound.

24. **(Currently amended)** The method of claim 23, wherein dose-response the dose-response curve for the translocation internalization measurement in the presence of the test compound is reduced as compared to the dose-response less than the dose-response curve for the translocation internalization measurement in the presence of the control compound.

25. **(Currently amended)** The method of claim 23, wherein the dose-response dose-response curve for the signaling measurement in the presence of the test compound is approximately equal to or greater than the dose-response dose-response curve for the signaling measurement in the presence of the control compound.

26. **(Currently amended)** The method of claim 24, wherein the reduced translocation internalization is determined by a decrease in the Max of the dose-response dose-response curve for the translocation internalization measurement in the presence of the test compound, as compared to

the Max of the dose-response dose-response curve for the translocation internalization measurement in the presence of the control compound.

27. **(Currently amended)** The method of claim 24, wherein the reduced translocation internalization is determined by an increase in the EC50 of the dose-response dose-response curve for the translocation internalization measurement in the presence of the test compound, as compared to the EC50 of the dose-response dose-response curve for the translocation internalization measurement in the presence of the control compound.

28-54. **(Canceled)**

55. **(Currently Amended)** A method of identifying a transmembrane receptor (TMR) G protein coupled receptor (GPCR) agonist, wherein the GPCR agonist TMR agonist (TMRA) is capable of activating TMR signaling while exhibiting reduced TMR internalization over a control compound, comprising the steps of:

- (a) providing a cell comprising at least one GPCR or a modified GPCR capable of activating intracellular signaling TMR, or a biologically active fragment thereof, wherein the cell further comprises and an arrestin, or a biologically active fragment thereof capable of binding a GPCR,
- (b) exposing the cell to at least one test compound,
- (c) measuring [[the]] GPCR signaling at one or more concentration of the test compound,
- (d) measuring the translocation internalization of the TMR GPCR at one or more concentration of the test compound,
- (e) quantitatively determining if the TMR GPCR internalization is reduced by comparing the TMR GPCR internalization in the presence of the test compound to the TMR GPCR internalization in the presence of a control compound, and wherein the GPCR signaling is activated in the presence of the test compound as compared to TMR GPCR signaling in the absence of the test compound, agonist; and
- (f) wherein reduced TMR a reduction in GPCR internalization in the presence of the test compound as compared to the control compound indicates the test compound is a GPCR

identifies a TMR agonist capable of activating GPCR signaling while exhibiting reduced GPCR internalization.

56. (Cancelled) The method of claim 55, wherein the TMR is a GPCR.

57. (Currently Amended) The method of claim 55, wherein the translocation internalization of the TMR GPCR is measured by monitoring localization of a detectable molecule bound to the arrestin or to the TMR GPCR.

58. (Previously Presented) The method of claim 55, wherein signaling is quantitated by measuring an intracellular effector, wherein said effector may be cAMP, cyclic GMP, calcium, a lipid, phosphatidylinositol, a hydrogen ion, or an ion transport molecule.

59 –61. (Cancelled).

62. (Currently Amended) The method of claim 55, wherein the signaling is activated for a longer time period after stimulation by the TMR test compound than the length of time of activation after stimulation by the control compound.

63. (Currently Amended) The method of claim 55, wherein the translocation internalization of the TMR GPCR is measured by determining the localization of the GPCR in the plasma membrane, pits, endosomes, endocytic vesicles, or cytosol.

64. (Currently Amended) The method of ~~claim 56~~ claim 55, wherein the GPCR is a class A, or class B receptor.

65. (Currently Amended) The method of ~~claim 56~~ claim 55, wherein the GPCR is a μ opioid, β_1 AR, β_2 AR, or dopamine receptor.

66. (Currently Amended) The method of claim 55, wherein the translocation internalization of the TMR GPCR is measured by visualization of a radioisotope, an epitope tag, an affinity label, an enzyme, a fluorescent group, or a chemiluminescent group attached to the arrestin or the TMR GPCR.

67. (Currently amended) The method of claim 55, wherein the signaling is measured at the same time as the translocation internalization is measured.

68. (Original) The method of claim 55, wherein the cell is exposed to the compound once, and wherein the cell is not exposed a second time to the compound.

69. **(Withdrawn)** A compound discovered by the method of claim 55.

70. **(Currently Amended)** The method of claim 55, wherein the ~~TMR~~ GPCR is a rat, mouse, pig, or primate ~~TMR~~ GPCR.

71. **(Currently Amended)** The method of claim 55, wherein the method is steps (a) – (f) are repeated, and wherein the ~~TMR~~ GPCR used in the repeated steps repeat method is from a different species than the GPCR used in the original method steps (a) – (f).

72. **(Currently Amended)** The method of claim 71, wherein a test compound that is used in steps (a) – (f) a TMRA in the original method is not used a TMRA in the repeated steps repeat method, and wherein the repeat method contains a TMR from a different species.

73. **(Previously Presented)** The method of claim 55, wherein the test compound is from a combinatorial library.

74. **(Original)** The method of claim 55, wherein the signaling in the presence of the test compound is approximately equal to or greater than the signaling in the presence of the control compound.

75. **(Currently amended)** The method of claim 55, wherein the method is repeated at different concentrations of compound to yield a ~~dose-response~~ dose-response curve for the signaling measurement and a ~~dose-response~~ dose-response curve for the ~~translocation~~ internalization measurement in the presence of the test compound.

76. **(Currently amended)** The method of claim 75, wherein the quantitative determination includes a comparison of the ~~dose-response~~ dose-response curve for the signaling measurement to the ~~dose-response~~ dose-response curve for the ~~translocation~~ internalization measurement.

77. **(Currently amended)** The method of claim 75, wherein a second ~~dose-response~~ dose-response curve for the signaling measurement and a second ~~dose-response~~ dose-response curve for the ~~translocation~~ internalization measurement are determined in the presence of control compound.

78. **(Currently amended)** The method of claim 77, wherein ~~dose-response~~ the dose-response curve for the ~~translocation~~ internalization measurement in the presence of the test compound is reduced as compared to the ~~dose-response~~ less than the dose-response curve for the ~~translocation~~ internalization measurement in the presence of, the control compound.

79. **(Currently amended)** The method of claim 77, wherein the ~~dose-response~~ dose-response curve for the signaling measurement in the presence of the test compound is approximately equal to or greater than the ~~dose-response~~ dose-response curve for the signaling measurement in the presence of the control compound.

80. **(Currently amended)** The method of claim 78, wherein the reduced ~~translocation~~ internalization is determined by a decrease in the Max of the ~~dose-response~~ dose-response curve for the ~~translocation~~ internalization measurement in the presence of the test compound, as compared to the Max of the ~~dose-response~~ dose-response curve for the ~~translocation~~ internalization measurement in the presence of the control compound.

81. **(Currently amended)** The method of claim 78, wherein the reduced ~~translocation~~ internalization is determined by an increase in the EC50 of the ~~dose-response~~ dose-response curve for the ~~translocation~~ internalization measurement in the presence of the test compound, as compared to the EC50 of the ~~dose-response~~ dose-response curve for the ~~translocation~~ internalization measurement in the presence of the control compound.

82-108 (Canceled)

109. **(Currently Amended)** The method of claim 16, wherein the primate ~~TMR GPCR~~ is a human ~~TMR GPCR~~.

110. **(Currently Amended)** The method of claim 70, wherein the primate ~~TMR GPCR~~ is a human ~~TMR GPCR~~.